Detection of DNA Damage Using Melting/Annealing Analysis Techniques

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ABSTRACT

A rapid and simple fluorescence screening assay for ionizing radiation, UV radiation-, chemical-, and enzyme-induced DNA damage is reported. This assay is based on a melting/annealing analysis technique and has been used to measure damage to calf thymus DNA. Damage resulting from low dose exposure to ionizing radiation, UV-C, DNAse I, and several classes of DNA damaging compounds can be measured.

INTRODUCTION

One of the approaches for reducing uncertainties in the assessment of human exposure is to better characterize the hazardous wastes that contaminate our environment. A significant limitation to this approach, however, is that sampling and laboratory analysis of contaminated environmental and biological samples, can be slow and expensive; thus, limiting the number of samples that can be analyzed within time and budget constraints. Faster and more cost-effective field screening methods can increase the amount of information available concerning the location, source and concentration of these pollutants.

Rapid and inexpensive indicator assays that can be used to screen for the genotoxicity of contaminated environmental samples and which can be related to a biological target (e.g., DNA) could be of significant benefit to the exposure assessment process. A variety of short term tests for genotoxicity/mutagenicity are currently being used to determine the extent of environmental hazards resulting from polluted water and sediments. Despite the description of short term, however, many of these assays are expensive to run, require sophisticated technical expertise, and are not well suited for potential field applications. The focus of this project is the characterization of rapid, sensitive and inexpensive assays for detection of damage to surrogate sequences of DNA caused by environmental pollutants and stressors. These methods are expected to provide the Agency with rapid, sensitive, and simple techniques that can be used among a panel of methods to determine the genotoxic potential of polluted samples.

METHODS

Figure 1. *Concept of Melting/Annealing Analysis*

This assay detects single strand breaks in target DNA as well as cyclobutane pyrimidine dimers, double strand breaks and intercalators. The concept for this assay is as follows: under certain conditions (i.e., high temperature or alkaline pH), double stranded DNA will unwind into single strands. Because temperature-induced unwinding (denaturation) occurs either more rapidly or under milder conditions if the DNA backbone has been broken along one of the strands, it can be used as an indicator of several characteristic types of DNA damage.

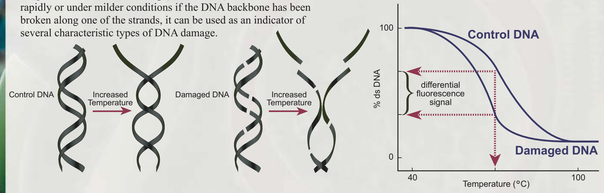


Figure 2. *PicoGreen: Indicator of Double Strand Structure*

The degree of DNA denaturation and annealing was determined using the double strand sensitive dye PicoGreen. This dye indicator dramatically increases its fluorescence in the presence of double strand (as opposed to single strand) DNA. This technique is very sensitive with a dynamic concentration range of 10 to 100 ng/mL for calf thymus DNA. For a 10 µL sample, the detection limit is 40 pg.

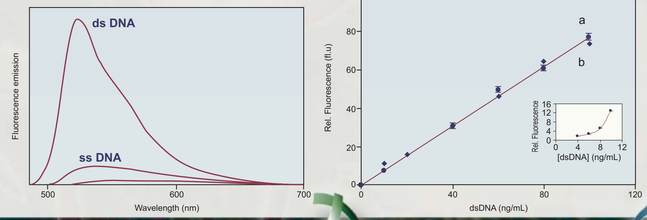
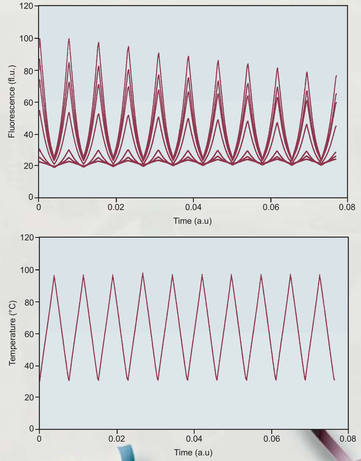


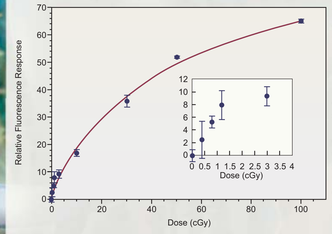
Figure 3. *Rapid Screening Format Using a Thermocycler-Fluorimeter*

The effect of various types of DNA damage on melting and annealing behavior was determined using a thermocyler-fluorimeter. The upper panel shows the relative amount of ds DNA reported by the fluorescence of PicoGreen using 0 to 100 ng/mL calf thymus DNA. The lower panel shows the temperature cycling profile.



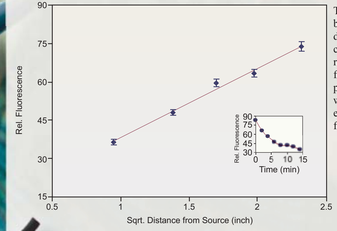
RESULTS

Figure 4. *Effect of Ionizing Radiation*



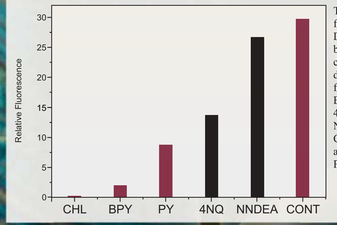
The relative fluorescence response of this assay is indicative of radiation-induced damage as a function of dose. The fluorescence response (% inhibition in fluorescence of PicoGreen) increased rapidly with increasing doses of radiation and began to saturate at dose levels above 10 cGy. The inset shows the low dose response. The detection limit (0.8 cGy) is similar to those shown by more complex and time-consuming methods such as capillary electrophoresis and alkaline unwinding assays. These data were collected using a standard thermocycler and fluorimeter.

Figure 5. *Effect of UV Radiation*



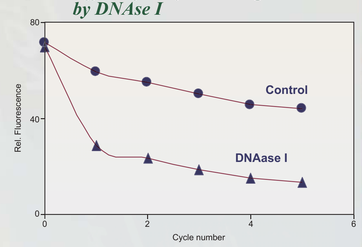
The peak fluorescence (at the beginning of the fifth cycle) decreased as a result of exposure of calf thymus DNA to UV-C (254 nm) radiation known to cause the formation of cyclobutane pyrimidine dimers. The response was dependent on both the time of exposure (inset) and the distance from the source.

Figure 6. *Effect of Chemically-Induced Damage*



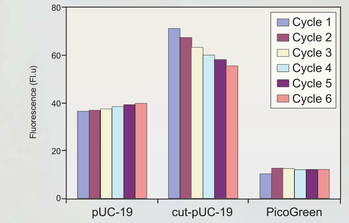
The initial fluorescence resulting from the binding of PicoGreen to ds DNA was, in some cases, inhibited by exposure of the DNA to compounds reported to cause damage. Abbreviations are as follows: CHL, Chloropyllin; BPY, Benzo(a)pyrene; PY, pyrene; 4NQ, 4-Nitroquinoline-N-oxide; NNDEA, N-Nitrosodiethylamine; CONT, Control. These compounds did not affect the fluorescence spectrum of PicoGreen in the absence of DNA.

Figure 7. *Effect of Enzymatic Degradation by DNAse I*



After treatment of the Calf Thymus DNA with DNAse I, the peak fluorescence response decreased compared to the control as a function of cycle number. The most dramatic change occurred after the first melting / annealing cycle.

Figure 8. *Effect of Restriction Enzyme Sau 3A*



After treatment of the supercoiled plasmid pUC-19 with the restriction endonuclease Sau 3A, the peak fluorescence response of the cycles as a group increased over the untreated plasmid DNA (cut-pUC-19). However, unlike the control sample, the fluorescence for the treated samples decreased (in an almost linear fashion) as a function of cycle number. This response could be repeated over numerous experiments and with two different supercoiled plasmids.

SUMMARY & CONCLUSIONS

The concept of Melting / Annealing analysis using PicoGreen as an indicator of ds DNA was used to develop a screening assay for DNA damage.

- The assay was rapid and simple.
- The assay responded to damage by several mechanisms including:
  - Ionizing Radiation (single strand breaks)
  - UV-C Radiation (cyclobutane pyrimidine dimers)
  - Chemical (intercalation, base modification)
  - Endonuclease (DNAse I, Sau 3A)
- Because temperature is readily controlled and fluorescence can be easily observed, the assay lends itself to development for sensor platforms.
- Future work will focus in more detail on the mechanisms by which the assay reports various types of DNA damage.

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